



Pettersson, I., Weeks, C., Norman, K., Knowles, T., & Nicol, C. (2017). Internal roosting location is associated with differential use of the outdoor range by free-range laying hens. *British Poultry Science*. <https://doi.org/10.1080/00071668.2017.1404007>

Peer reviewed version

Link to published version (if available):  
[10.1080/00071668.2017.1404007](https://doi.org/10.1080/00071668.2017.1404007)

[Link to publication record in Explore Bristol Research](#)  
PDF-document

This is the author accepted manuscript (AAM). The final published version (version of record) is available online via Taylor & Francis at <https://www.tandfonline.com/doi/full/10.1080/00071668.2017.1404007> . Please refer to any applicable terms of use of the publisher.

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1    **Internal roosting location is associated with differential use of the**  
2    **outdoor range by free-range laying hens**

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## 25    **Abstract**

- 26        1. In commercial free-range systems for laying hens popholes to the outdoor range are  
27            often installed on one side of the house only. In multi-tier systems, it is possible that  
28            some individuals fail to access the range due to internal barriers to movement.
- 29        2. Five commercial multi-tier flocks from different units were studied. For each flock,  
30            two different colour markers were used to distinguish 200 birds roosting near the  
31            popholes (NP-Roost) and 200 birds roosting far from the popholes (FP-Roost) at  
32            night. The following day, counts of marked birds on the range and inside the house  
33            were performed.
- 34        3. Significantly more NP-Roost birds were observed in all areas of the outdoor range  
35            than FP-Roost birds the next day. Distance of FP area from the popholes was very  
36            strongly positively correlated with effect size in the adjacent range area.
- 37        4. Additionally, in the indoor area far from the popholes (FP) more FP-Roost birds  
38            were observed the next day than NP-Roost birds. In the indoor area near to the  
39            popholes (NP) more NP-Roost birds were observed the next day than FP-Roost  
40            birds.
- 41        5. These results suggest that roosting location is associated with differential range use  
42            when popholes are only available on one side of the shed as birds that roosted far  
43            from the popholes used the range less.

44

## 45    **Keywords**

46    Welfare, Behaviour, Laying hens, Multi-tier, Aviary, Range use, Free range

47

## 48    **1. Introduction**

49 Loose-housing systems for laying hens allow birds to move around the house freely,  
 50 accessing various resources such as litter, feed, water, nestboxes, and in free-range systems,  
 51 the outdoor range. In both single and multi-tier (also known as aviary) systems the feed,  
 52 water and nestboxes are on one or more elevated tiers with litter and range access available  
 53 at ground level. Questions have been raised about the ability of birds to move throughout  
 54 these systems, particularly where multiple potential barriers to movement are present  
 55 (Stratmann *et al.*, 2015; Ali *et al.*, 2016). In all loose-housing systems birds have to  
 56 negotiate level changes (from tier to ground, or between tiers) to access resources, and some  
 57 housing configurations require birds to negotiate level changes just to move from one side of  
 58 the house to the other. Research has shown behavioural signs of hesitation and difficulty  
 59 negotiating the key level change between the slats (or first tier) and the litter (Pettersson *et*  
 60 *al.*, 2017) and ramps between all levels of a multi-tier system were found to reduce falls and  
 61 collisions (Stratmann *et al.*, 2015).

62 An additional potential barrier to free movement can arise if hens crowd together, creating  
 63 increased stocking densities in certain areas, particularly around resources (Collins *et al.*,  
 64 2011; Lentfer *et al.*, 2013) and walls (Newberry and Hall, 1990). Higher stocking densities  
 65 have been associated with reduced bird movement (Appleby *et al.*, 1989; Carmichael *et al.*,  
 66 1999).

67 At night, hens choose to roost on high perches when available (Olsson and Keeling, 2000)  
 68 and in multi-tier systems will fill the higher tiers (Odén *et al.*, 2002; Ali *et al.*, 2016). A  
 69 study of groups (mean group size of 588 birds) housed in a multi-tier system found that birds  
 70 that roosted in end areas of the pen stayed within that area during the following days more  
 71 than would be expected by chance and often roosted in the same place in the following night  
 72 (Odén *et al.*, 2000). In a few small experimental studies hens have shown individual  
 73 differences in location use within commercial-style aviaries (Freire *et al.*, 2003; Campbell *et*  
 74 *al.*, 2016) although it is not clear whether this was due to capability of moving around,  
 75 individual preference or feather pecking by other birds. Home ranges and ‘activity centres’

76 can be calculated for individual laying hens within commercial units (Leone and Estevez,  
 77 2008; Rodriguez-Aurrekoetxea and Estevez, 2016) suggesting that birds tend to use certain  
 78 areas more often. Interestingly birds that range more were found to have larger home ranges  
 79 and activity centres (Rodriguez-Aurrekoetxea and Estevez, 2016), possibly because of the  
 80 increased opportunity to travel further in the outdoor area. Inside the house having a small  
 81 home range may not be an issue as all key resources such as feed, water, nestboxes and litter  
 82 are usually well distributed throughout. However, range access may prove difficult in this  
 83 case.

84 The proportion of birds using the range at a given time is often low (Pettersson *et al.*, 2016a)  
 85 and research using RFID tracking technology on commercial farms has found that some  
 86 birds do not appear to use the popholes, and therefore the range, at all (8%: Richards *et al.*,  
 87 2011; 29.5%: Gebhardt-Henrich *et al.*, 2014). Some consumers believe free-range hens to  
 88 be happier and healthier than in other systems and access to the range to be the most  
 89 important factor for welfare (Pettersson *et al.*, 2016b). Actual levels of range use in  
 90 commercial systems may therefore not meet consumer expectation. When popholes are  
 91 available only on one long side of the house, some birds will have to travel many metres to  
 92 access the range and in multi-tier systems a view of the popholes is blocked by the tiers  
 93 themselves for birds in most areas of the house. It is possible that hens may not even be  
 94 aware of the range area if they cannot see the popholes. If this was the case we would expect  
 95 birds that started off the day in an area where popholes were visible to be more likely to use  
 96 the range than those that have to travel far to access that area.

97 The two aims of this study were to test our predictions that (i) birds that roost near to the  
 98 popholes will be more likely to use the range area the next day than those that roost far away  
 99 from the popholes and (ii) birds will stay near to their night-time roosting location the next  
 100 day.

## 101 **2. Materials and Methods**

102 The study used five commercial free-range multi-tier flocks with flock sizes of  
 103 approximately 16,000 birds. Two flocks were on the same farm but housed in separate  
 104 buildings (see the table for flock and house information). All flocks had pre-existing colony  
 105 divisions within the house separating the flock into four colonies of approximately 4000  
 106 birds and popholes were fitted to one long side of the house only, evenly spaced. Birds were  
 107 not able to access other colonies areas inside the house although they could when out on the  
 108 range in all flocks except for flock 1 which had physical colony divisions on the range. Fig 1  
 109 shows the layout of multi-tier stacks within flocks 2-5. Flock 1 was slightly different as the  
 110 shed was divided into colonies in a 2x2 design. Nonetheless, the layout of stacks within the  
 111 studied colony remains the same as the other flocks. Although some houses closed the area  
 112 under the tiers off during the night, these were not closed off for flocks 1,3,4 and 5 during  
 113 the day. This meant that birds could walk along the litter from one side of the house to the  
 114 other. In flock 2 the area under the tiers was closed off for the first of the three observations  
 115 as the producer did not choose to open up this area until 11am. All flocks were allowed out  
 116 onto the range at 9 am. All flocks had 16-17 hours light.

117 The study involved a 2-day visit to each flock when the birds were 41-47 weeks of age.  
 118 These visits took place between late-August and mid-November 2016 and weather  
 119 conditions were similar for all flocks. House design and dimensions were recorded. Ethical  
 120 permission was obtained from the University of Bristol ethical committee prior to starting  
 121 the project.

122 --- SUGGESTED LOCATION FOR TABLE ---

## 123 **2.1. Marking birds**

124 In order to establish whether birds used all areas of the house and range, two samples of  
 125 individual hens were marked during the night of Day 1 (between 9pm and 2am) when the  
 126 lights were off and birds were roosting. Researchers used red light head torches when in the  
 127 house to minimise bird disturbance. One colony was selected per flock and 400 birds

128 (approximately 10% of the colony) were marked. Two distinct areas were established –  
 129 ‘near’ the popholes (NP) and ‘far’ from the popholes (FP) (see fig 1). On the tiers, 200  
 130 roosting birds from the NP area were marked green (NP-Roost) and 200 roosting birds from  
 131 the FP area were marked pink (FP-Roost). Birds were selected evenly across the areas, with  
 132 the highest tier unable to be sampled due to accessibility. All birds were generally in good  
 133 condition although any birds with visible problems such as bumblefoot were excluded.  
 134 Livestock marker crayons (Paintstik®, All-Weather®, USA) in fluorescent green and  
 135 fluorescent pink were used to colour one entire leg of each bird. The two colours plus  
 136 another (blue) were tested in a pilot trial and blue was found to be the least visible so was  
 137 not used. One researcher lifted each bird from its roosting position and held it steady while  
 138 another researcher marked the leg with the appropriate colour. Each bird was then placed  
 139 back in the same location, where they remained, with little indication of disturbance. This  
 140 process took about 30 seconds per bird.

## 141 **2.2. Observations and counts**

142 Marked birds were counted at three time-points the next day (approximately 90min apart)  
 143 between 10am and 1pm. Mean temperature, relative humidity and light levels were similar  
 144 in both NP and FP areas. Indoor counts were performed first at each time point, immediately  
 145 followed by range counts. Further counts were not performed; in part owing to time  
 146 constraints but also because the researcher’s presence was likely to be disturbing the natural  
 147 distribution of the birds with every observation. When assessing range use the number of  
 148 NP-Roost and FP-Roost marked birds were counted in two areas of the range; adjacent and  
 149 non-adjacent. The adjacent area was divided from the non-adjacent area by visualising a line  
 150 as a continuation of the internal colony divisions (see fig 1). The observer had experience  
 151 counting ranging birds and walked methodically through the range areas counting both the  
 152 total number of birds outside, and those that were marked. Although only flock 1 had  
 153 physical colony divisions on the range we hypothesised that more marked birds would be  
 154 seen within the area adjacent to the studied colony popholes, hence dividing the range area

up visually for these counts. It was too difficult to see the legs of birds on the litter area of the house so these birds were not included in the counts. The researcher walked along the edge of the stacks in the NP area of the house, counting all NP-Roost and FP-Roost marked birds visible on the tiers in this section (back of top tier excluded). A head torch was used to clearly see birds further back on the tiers. Not all birds were visible (e.g. within nestboxes and on the back of the top tier) and these were not counted as it was deemed to be too disruptive for the researcher to look in nestboxes or climb the tiers. The same was then repeated for the FP area. It was not possible for the researcher to be blinded to the groups marked but as the count measure is objective it is unlikely that bias occurred.

--- SUGGESTED LOCATION FOR FIGURE 1 ---

### 2.3. Statistical analysis

The multilevel statistics package MLwiN (Charlton et al. 2017) was used for the statistical analyses to accommodate the doubly repeated measures of measures within observation number, within house. A separate multi-level model was produced for each area where counts were taken – on the range (adjacent and non-adjacent) and in the house (NP and FP). To look for potential differences in the numbers of NP-Roost and FP-Roost birds, the difference between the two counts was calculated (FP-Roost minus NP-Roost) and used as the outcome variable in these models. Differences in observation number was also tested within these models as a fixed effect. The residuals from the models were checked to ensure they met the assumptions of the model. Although the differences between counts were used in the model, mean counts have been reported for clarity.

For observation 1 in flock 2, the area under the litter was blocked off. As this may have had a large effect on the results and did not match the other flocks, this value has been replaced with an estimated value. The estimation was produced by averaging the values for observations 2 and 3.



180 To check for correlations between distance of FP area from a pophole and size of the effect  
 181 on range use, the number of FP-Roost birds counted on the range was subtracted from the  
 182 number of NP-Roost birds counted on the range for each flock. After checking for  
 183 normality, Pearson correlations were performed.

184

### 185 **3. Results**

#### 186 **3.1. Use of outdoor range areas**

187 On average across all flocks and observations 7.34% of the whole flock (both marked and  
 188 unmarked birds) were seen out on the range at a time with low variability (range of means  
 189 between flocks: 6.03-8.98%, range of means across the three observations: 7.23-7.42). On  
 190 average, 5.38% (SD=2.60) of marked birds were seen out on the range at a time.

191 Within the adjacent range area, significantly more NP-Roost birds (mean=16.20, SD=8.10)  
 192 were counted than FP-Roost birds (5.33, SD=3.14) (parameter estimate: -9.733(SE: 2.553),  
 193  $p<0.001$ ). There was no significant effect of observation number. See fig 2a. Within the non-  
 194 adjacent range area significantly more NP-Roost (11.47, SD=7.67) than FP-Roost birds  
 195 (4.00, SD=3.60) were also observed (parameter estimate: -6.200(SE: 2.293),  $p<0.001$ ) with  
 196 no effect of observation number (Fig 2b).

197 Distance of FP area from the popholes was very strongly positively correlated with effect  
 198 size ( $r=0.988$ ,  $n=5$ ,  $p=0.002$ ). As this distance increased, the proportion of NP-Roost birds  
 199 relative to FP-Roost birds, seen on the range also increased. For the non-adjacent area of the  
 200 range this relationship did not reach significance ( $r=0.816$ ,  $n=5$ ,  $p=0.092$ ).

201 --- SUGGESTED LOCATION FOR FIGURE 2 ---

#### 202 **3.2. Use of NP and FP areas in the house**

203 Within the FP area of the house significantly more FP-Roost birds (15.86, SD=6.16) were  
 204 counted than NP-Roost birds (6.93, SD=4.07) (parameter estimate: 7.200 (SE: 1.865),  
 205  $p < 0.001$ ) (see Fig 3b). There were significant differences between observations with mean  
 206 differences between FP-Roost and NP-Roost birds for observations 1-3 at 7.20 (SD: 2.588),  
 207 10.20 (SD: 5.02) and 3.40 (SD: 5.77) respectively.

208 In the NP area of the house significantly more NP-Roost (11.20, SD=4.04) birds than FP-  
 209 Roost birds (6.80, SD=2.12) were counted (parameter estimate: -3.133 (SE: 0.810),  $p < 0.001$ )  
 210 (see Fig 3a). There was no effect of observation number.

211 --- SUGGESTED LOCATION FOR FIGURE 3 ---

212

## 213 4. Discussion

214 The results suggest that night time roosting location affects the next day's range use by  
 215 individual birds in free-range flocks. As most marked birds should be found in the adjacent  
 216 area of the range we expected counts from this area to be the most likely to show any  
 217 significant effects. The strongest results were seen in the adjacent area with more than twice  
 218 the number of birds that roosted near the popholes (NP-Roost birds) seen in this outside area  
 219 than birds that roosted far from the popholes (FP-Roost birds) across all three observations.  
 220 Significantly more NP birds than FP-Roost birds were also observed in the non-adjacent  
 221 area, suggesting that this effect of bird roosting location on range use exists even in less  
 222 accessible areas of the range.

223 These results suggest that by allowing access to outdoor areas on one side of a laying house  
 224 only, some of the birds may have limited access to the outside compared with others simply  
 225 because of their location in the house. The reasons why roosting far away from a pophole  
 226 reduces the likelihood of using the range cannot be determined from this study, although  
 227 there are a number of possible explanations. Hens appear to have individual home ranges in

228 commercial units (Rodriguez-Aurrekoetxea and Estevez, 2016) and this may limit their  
229 outdoor range use if a pophole is not available within this home range. It may also be the  
230 case that it is important for popholes to be visible for a bird to choose to use the range area.  
231 Another possible explanation is that the birds which roost in certain areas of the house may  
232 have some other characteristics affecting their use of the range such as weight, or  
233 behavioural traits such as fearfulness. However, this remains speculation at the moment and  
234 has not been tested. The fact that the difference in numbers of birds from both roosting  
235 locations was greater on the range when the distance to travel was greater suggests that the  
236 results seen in this study are closely related to the distance of roosting location from  
237 available popholes.

238 Our second objective was to test the prediction that hens stay in the vicinity of their roosting  
239 location the next day. This study found that more FP-Roost birds than NP-Roost birds were  
240 observed in the FP house area the next day and more NP-Roost than FP-Roost birds were  
241 observed in the NP house area. These results suggest that birds tend to stay in the same area  
242 as they roosted the night before, with few birds coming from other areas of the house. This is  
243 backed up by the results for the range areas, as birds with popholes near to their roosting  
244 location (NP-Roost) birds ranged more. If hens are reluctant to travel far from their roosting  
245 location the FP-Roost birds are less likely to make it to a pophole and out onto the range.  
246 There was an effect of observation number in the FP area. Although the direction of the  
247 effect remained consistent across all observations for more FP-Roost birds, the size of this  
248 effect increased for the second observation and then decreased for the final observation  
249 suggesting that the numbers of birds had begun to even out by observation three. However,  
250 this preliminary study looked only at a few hours following pophole opening and so  
251 information on bird movement over a longer period is essential to establish if this effect is  
252 true.

253 This study was designed to provide the first evidence of a problem often discussed by  
254 producers and scientists but lacking in scientific evidence – that some laying hens do not

255 access certain resources, particularly the outdoor range where popholes are limited. Very  
256 little work has studied the effect of bird roosting location on movement in commercial flocks  
257 although some data is available (Odén *et al.*, 2000) and this is the first to look at the effect  
258 on multiple free-range commercial units.

259 As this was a preliminary study, further measures that may have helped to determine the  
260 specific reasons behind the results such as measures of individual health and behaviour were  
261 not taken but would be a valuable avenue for further investigation. Additionally, this study  
262 only looked at one day and did not cover whether birds return to the same area to roost. This  
263 was mostly due to limitations of the marking method as it was not designed to last longer  
264 than a day or two. The results of this study did not find that marked bird numbers in each  
265 location were affected by the time observed in most areas (within the scope of the study) but  
266 it would be useful to know if this is a short or long-term effect. Due to practical and safety  
267 reasons, it was not possible to mark birds from the highest tier. It is possible that this may  
268 have influenced the results as birds that perch on the highest tier may have different  
269 behavioural traits than the rest of the flock.

270 The marking method trialled in this study was found to be relatively successful. It was easy  
271 to mark birds with two people and the colours chosen were very distinct. No negative effects  
272 such as feather pecking by conspecifics were seen by the researchers during the study or  
273 reported by the producers, likely because the legs of the birds were marked rather than the  
274 plumage. While leg marks were easy to see on tiers, they were difficult to spot on crowded  
275 ground areas such as the litter. For the purpose of this study this was not considered a major  
276 issue as information about bird movement and location could still be collected from other  
277 birds. The method may not be appropriate however if litter use is of particular interest. On  
278 average, a slightly lower percentage of marked birds were seen ranging than the total  
279 percentage of marked and unmarked birds ranging. As this was only a difference of  
280 approximately 2% the ability of the researcher to identify marked birds on the range was

adequate. The method appears to be useful for marking groups of birds (rather than individuals) on a short-term basis in commercial flocks.

To conclude, this study provides the first evidence that some laying hens within large free-range commercial units may have limited access to the outdoor range area if popholes are provided on one side of the house only. Efforts to improve the design of free-range units are often focused on the range area or pophole size and number. This research has highlighted a need to consider the placement of popholes in addition to these factors in order to provide access to all resources for all individuals in a commercial flock.

## Acknowledgements

The authors gratefully acknowledge funding by Noble Foods and would like to thank the four producers that kindly allowed their flocks to be studied.

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Flock number	Flock size	Genotype	System	Number of tiers	Light schedule	Feeds per day	Number of popholes (studied colony)	Pophole size (height x width) (cm)	Distance of FP area from popholes (m)	Distance of NP area from popholes (m)	No. of tier stacks to cross (from FP to NP)	Bird age at visit (weeks)
1	15837	Lohmann Brown	Jansen	3	6:00-22:00	5	6	46 x 230	9.65	4	2	42
2	16032	Lohmann Brown	Dutchman Natura Twin	2	5:15-21:40	5	5	50 x 272	12.10	3.7	2	41
3	16032	Bovan Brown	Dutchman Natura Twin	2	6:00-21:00	5	5	49 x 268	12.10	3.1	2	47
4	16032	Bovan Brown	Dutchman Natura Twin	2	6:00-21:00	5	5	50 x 270	12.80	3.35	2	47



349

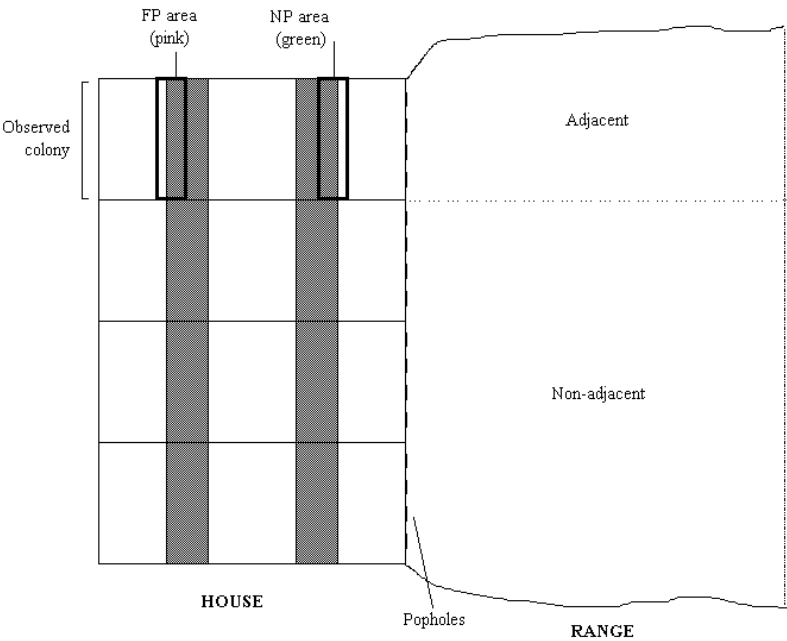
350

5	16032	Lohmann Brown	Vencomatic Veranda Aviary	2	5:00- 22:00	6	6	51 x 205	9.80	2.6	2	44
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351 Figure 1: Diagram of an example house layout with observation areas marked.

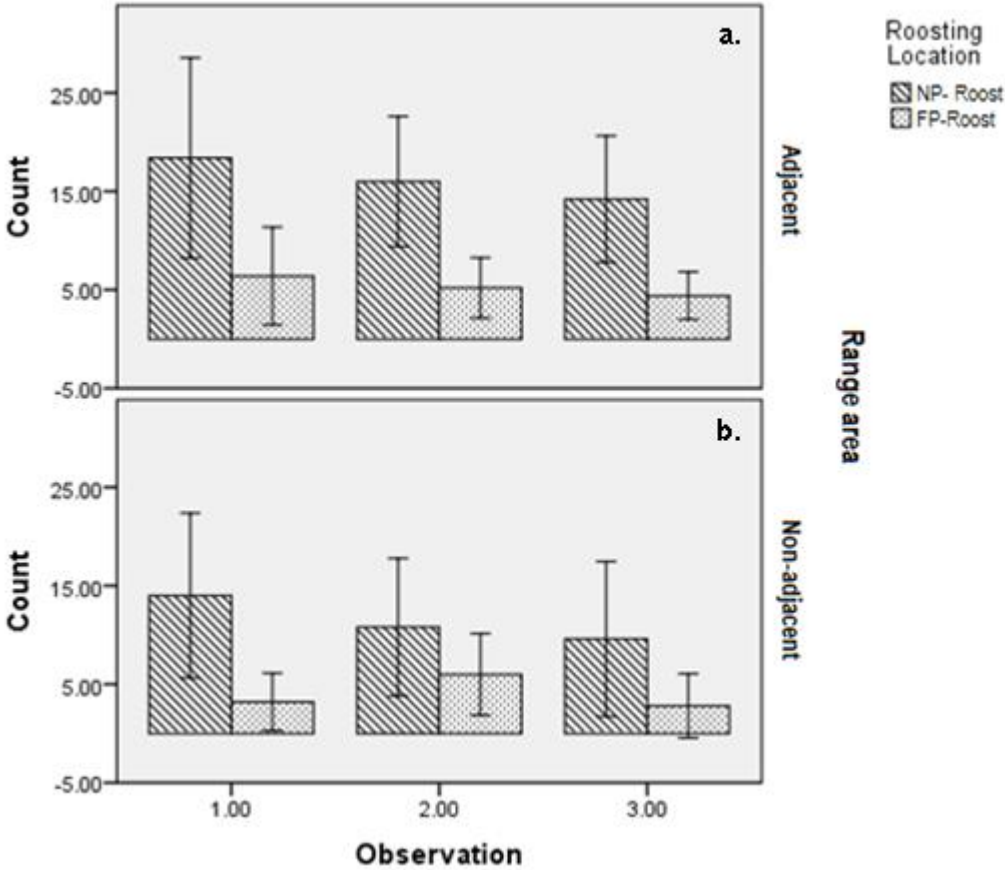
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354 Figure 2: Mean counts of marked birds in the two range locations across the three  
355 observations (error bars:  $\pm 2SE$ ).

356



357 Figure 3: Mean counts of marked birds in the two indoor locations across the three  
358 observations (error bars:  $\pm 2SE$ ).

